

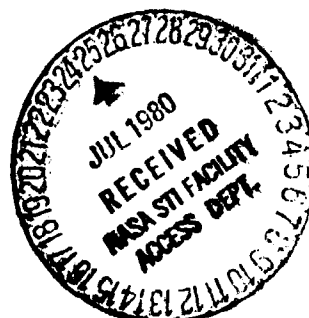
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THE EFFECT OF HYPODYNAMIA ON MINERAL AND PROTEIN METABOLISM  
IN CALCIFIED TISSUES OF THE MAXILLODENTAL SYSTEM  
(EXPERIMENTAL RADIOISOTOPE STUDY)

A. A. Prokhonchukov, Ye. A. Kovalenko, A. G. Kolesnik,  
Yu. I. Kondrat'yev, and N. A. Ilyushko

Translation of "Vliyaniye gipodinamii na mineral'nyy i belkovyy sbmen  
v obyazvestvlennykh tkanyakh zubochehyustnoy sistemy (eksperimental'noye  
radioizotopnoye issledovaniye)," Stomatologiya, Vol. 49, No. 4, 1970,  
pp. 1 - 6



(NASA-TM-75998) THE EFFECT OF HYPODYNAMIA  
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CALCIFIED TISSUES OF THE MAXILLODENTAL  
SYSTEM (EXPERIMENTAL RADIOISOTOPE STUDY)  
(National Aeronautics and Space

N80-27078

Unclas  
G3/52 27945

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION  
WASHINGTON, D.C. 20546  
JULY 1980

## STANDARD TITLE PAGE

1. Report No. <b>NASA TM-75998</b>	2. Government Accession No.	3. Recipient's Catalog No.	
4. Title and Subtitle <b>THE EFFECT OF HYPODYNAMIA ON MINERAL AND PROTEIN METABOLISM IN CALCIFIED TISSUES OF THE MAXILLODENTAL SYSTEM</b>		5. Report Date <b>July 1980</b>	6. Performing Organization Code
7. Author(s) <b>A. A. Prokhonchukov, Ye. A. Kovalenko, A. G. Kolesnik, Yu. I. Kondrat'yev, and N. A. Ilyushko.</b>		8. Performing Organization Report No.	10. Work Unit No.
9. Performing Organization Name and Address <b>Leo Kanner Associates Redwood City, California 94063</b>		11. Contract or Grant No. <b>NASW - 3199</b>	13. Type of Report and Period Covered <b>Translation</b>
12. Sponsoring Agency Name and Address <b>National Aeronautics and Space Administration, Washington, D. C. 20546</b>		14. Sponsoring Agency Code	
15. Supplementary Notes <b>Translation of "Vliyaniye gipodinamii na mineral'nyy i belkovyy obmen v obyzzvestvlennykh tkanyakh zubochehyustnoy sistemy (eksperimental'noye radioizotopnoye issledovaniye)," Stomatologiya, Vol. 49, No. 4, 1970, pp. 1 - 6</b>			
16. Abstract <b>Mineral and protein metabolism was studied in experiments on 60 white rats, using P<sup>32</sup> and Ca<sup>45</sup> uptake in the mineral fractions, 2C<sup>14</sup>-glycine in the protein fractions, and P<sup>32</sup> in both fractions of calcified tissues as indices over a 100-day period of experimental hypodynamia. Combined alterations in mineral and protein metabolism occurred in the calcified tissues of the experimental animals. The most pronounced changes were found in P<sup>32</sup> and 2C<sup>14</sup>-glycine metabolism. In the incisors and femoral bones, these alterations occurred in two phases: P<sup>32</sup> and 2C<sup>14</sup>-glycine uptake first increased, then decreased. Changes in Ca<sup>45</sup> metabolism were less pronounced, particularly in the initial period of the experiment. A marked reduction in P<sup>32</sup>, Ca<sup>45</sup>, and 2C<sup>14</sup>-glycine uptake was found in various fractions of the calcified tissues on the 100th day of experimental hypodynamia.</b>			
17. Key Words (Selected by Author(s))		18. Distribution Statement <b>Unclassified-Unlimited</b>	
19. Security Classif. (of this report) <b>Unclassified</b>	20. Security Classif. (of this page) <b>Unclassified</b>	21. No. of Pages <b>15</b>	22. Price

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THE EFFECT OF HYPODYNAMIA ON MINERAL AND PROTEIN METABOLISM  
IN CALCIFIED TISSUES OF THE MAXILLODENTAL SYSTEM  
(EXPERIMENTAL RADIOISOTOPE STUDY)

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In recent years, many researchers have focused their attention on /1\* various physiological disorders caused by restriction of the body's motor functions: hypodynamia (Deitrick et al., 1948; Krows and Raab, 1961; Groveline, 1962; Giovani et al., 1964; Lamb, 1964; Hatter and McMillan, 1968; V. V. Perin et al., 1967; V. V. Portugalov et al., 1967; A. V. Korobkov, 1968; L. I. Kakurin, 1968, etc.).

The problem of hypodynamia is closely linked to an extremely wide /2 range of problems in modern biology and medicine (including dentistry). We include here problems associated with restriction of motor activity for persons performing mental work and production tasks (machine operators, etc.) and disorders arising in patients confined to prolonged bed rest (or having immobilized limbs or lower jaws); rehabilitation procedures; medical and biological aspects of high altitude flight in high-speed aircraft, space flight, submarine cruises, etc.

The study of hypodynamia has now become especially important because of its association with space flight, during which a complex of various intrinsically different extreme factors acts on the body: G-forces, weightlessness, hypodynamia, cramped quarters, cabin atmosphere peculiarities, elevated cabin gas ionization, possible cabin decompression, dietary irregularities, the effects of ionizing radiation, and a number of neuropsychiatric factors.

The extreme factors of space flight may have both direct and mediated effects on the maxillo dental system. Ionizing radiation, for example,

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\*Numbers in the margin indicate pagination in the foreign text.

may cause specific radiological changes in the maxillo-dental system (Cerne and Bataille, 1947; English, et al., 1955; A. I. Rybakov, 1956; Geisford and Rueckert, 1956; Yu. N. Uspenskiy et al., 1957; Niebel and Ninan, 1957; A. A. Prokhonchukov, 1957, 1960, 1964; A. A. Prokhonchukov, V. V. Panikarovskiy, 1962; Dadson, 1962; A. A. Prokhonchukov, 1964; G. M. Barer, 1962, 1965; A. A. Prokhonchukov, V. V. Panikarovskiy, N. A. Zhizhina, 1966, etc.). Ionizing radiation also intensifies the formation of caries and atrophy of the alveolar processes of the maxillae (Ershoff and Bavetta, 1958; Yu. A. Fedorov, 1961, 1963; A. A. Prokhonchukov, 1964; G. M. Barer, 1965, etc.).

The microflora in the atmosphere and body (among them those of the digestive tract) may be altered, and their immunological reactivity may also decrease (Weber, 1964; A. V. Lebedinskiy et al., 1966; G. P. Mikhailovskiy et al., 1967). The role of oral microflora in the genesis of various pathogenic processes in the maxillo-dental system is widely known (L. N. Rebreyeva, V. F. Kuskova, 1967). Halitosis frequently occurs during confinement to cramped quarters (Yu. A. Fedorov, 1967).

An excess of positively-charged atmospheric ions harmful to the body, including its maxillo-dental system (A. A. Minkh, 1967, etc.) may accumulate within the limited confines of the cabin (V. V. Ogleznev, 1962, 1965; A. A. Minkh, 1967, etc.).

Possible drops in cabin atmospheric pressure, hypoxia, and other factors involved in high altitude flight in aircraft and space craft may engender odontalgias (M. K. Geykin, 1949; Groizdek and Horodyshi, 1963; Yu. A. Fedorov, 1967, etc.) or facilitate the development of parodontopathies (M. K. Geykin, 1950; I. O. Novik, 1964; Yu. A. Petrovich and R. P. Podorozhnaya, 1965; S. K. Kotlyar, 1967; N. F. Danilevskiy, 1968; A. I. Matveyeva, 1968, etc.) and other pathological processes in the maxillo-dental system (M. K. Geykin, 1950; A. S. Bobrik, 1968, etc.).

The use of soft foods on space flights (Taylor, 1956; N. M. Sisakyan et al., 1962; Bovee et al., 1963; Nanz, 1964, etc.) may in some cases interfere with the self-cleansing processes of the oral cavity, and

masticatory stimulation of the parodontia (particularly during weightlessness) is reduced, which in turn may bring about pathologies (McCall and Szmyd, 1964; Hartby, 1965; Shalit, 1966, etc.).

We are all familiar with the fact that alterations of neuro-endocrine regulation in the body may be conducive to various forms of parodontopathy (E. D. Bromberg, 1967, 1968; N. F. Danilevskiy, 1968, etc.). These same factors may also have an effect on the hard tissues of the teeth (A. A. Prokhonchukov, N. A. Zhishina, 1967, etc.). Changes in the hard tissue of the tooth may appear not only as caries, but also as hyperesthesia of the enamel, which apparently is initially caused by high G-forces, but then becomes associated with disorders of mineral metabolism consequent to weightlessness and hypodynamia during space flight (McCall and Szmyd, 1964; Hartley, 1965; Shalit, 1966; Yu. A. Fedorov, 1967, etc.).

Together with weightlessness, hypodynamia is one of the most intrinsically important of the various factors of space flight. It obviously may have a rather serious effect on the whole body, and on metabolic processes in particular. Hypodynamia (and, obviously, weightlessness) is initially characterized by disorders primarily of mineral (especially calcium) metabolism in both the skeleton and the body as a whole (Brannon et al., 1963; Miller et al., 1964; Stevens, 1966; Lynch and Jensen, 1967; Hatter and McMillan, 1968).

Following a 2 month stay in hypodynamic conditions there is reduced mineralization of human skeletal osseous tissues (Ye. N. Biryukov and L. I. Kakurin, 1966; Ye. N. Biryukov, 1968; L. I. Kakurin, 1968). A decrease in extremity bone osseous tissue mineralization is found following prolonged immobilization (Deitrick et al., 1948; Lynch et al., 1967; Hatter and McMillan, 1968).

There are isolated reports in the literature describing parodontopathies in animals subjected to restricted mobility (A. A. Prokhonchukov, N. A. Zhishina, 1967). Hypodynamia also disturbs the secretory functions of the stomach (Brodie et al., 1962).

Elevated blood calcium content, increased calcium excretion in the urine and feces, and reduced mineralization in certain parts of the skeletal bones were noted in American astronauts who completed space flights of up to 2 weeks duration in the "Gemini" series of space craft (Hatter and McMillan, 1968). Similar alterations were discovered in the dogs "Veterok" and "Ugolek" following a 22-day flight in the Soviet "Cosmos-110" biosatellite (Ye. N. Biryukov, 1968).

These factors provide some basis for the assertion that, resulting from a pattern of extreme factors acting during space flight, a definite background is created for the development of pathological processes in the maxillo dental system (hypoplasia of the tooth enamel, dental caries, parodontopathy, diseases of the oral cavity mucous membranes, etc.).

It is important to emphasize that the effects of almost all of the extreme factors of space flight are closely associated (directly or indirectly) with disturbances of body metabolic processes, among them calcium metabolism, disorders of which, as previously indicated, are of considerable significance. Metabolic disorders, including disorders of calcium metabolism, are also of material importance in the pathogenesis of various pathological processes in the maxillo dental system (A. A. Prokhonchukov and N. A. Zhizhina, 1967).

For study of the effects of space flight factors on metabolic processes in the calcified tissues of the maxillo dental system, we chose one of the most important (and most capable of simulation under terrestrial conditions) factors -- hypodynamia. At least two unanswered questions arose during our analysis of available reference data: what is the nature of the developmental dynamics of disorders in mineral (calcium) metabolism during hypodynamia, and are these disorders associated with alterations in protein metabolism? Combined study of mineral and protein metabolism in calcified tissues during experimental hypodynamia was a part of the goal of our research. We proceeded from the assumption (working hypothesis) that disorders of mineral (calcium) metabolism are closely linked to  $P^{32}$  metabolism and to changes in protein metabolism, since combined disorders of mineral and protein metabolism are character-

istic for various kinds of calcified tissue pathologies (A. A. Prokhonchukov, 1964; A. A. Prokhonchukov, N. A. Zhizhina, 1967). We did not encounter any similar research in the available literature.

### Materials and Methods

In order to answer the questions we posed, hypodynamic states were created in experiments on 60 rats (of which 20 were controls): the animals were placed in specially-constructed cages which restricted their mobility. Experimental and control animals received needed nutriment and water in sufficient quantities. To study the dynamics of metabolic processes, studies were performed during 15, 30, 60, and 100 days of experimental hypodynamia. Mineral and protein metabolisms were studied using radioisotope methods described by A. A. Prokhonchukov (1957, 1961) and indices of  $P^{32}$  and  $Ca^{45}$  uptake in the mineral fraction (MF) and  $2C^{14}$ -glycine and  $P^{32}$  uptake in the protein fraction (PF) of the teeth (molars and incisors) and bones (maxillary and femoral).

We should mention that the maxillo-dental system in the rat is an extremely valuable subject for the study of various metabolic disorders of calcified tissues. The constantly-growing rodent incisors are like a constantly-acting ontogenetic model of the formation and calcification of protein matrices; they are quite sensitive to various changes in metabolic processes. The molars of the rat are, conversely, characterized by the lowest level of metabolic processes among all the body's tissues and are extremely resistant to differing metabolic shifts. The inferior maxillary bone is characterized by slower metabolic processes or a higher degree of mineralization (A. A. Prokhonchukov, 1964; N. A. Zhizhina and A. A. Prokhonchukov, 1965, 1967). Moreover, the lower jaw retains its usual mobility, even when food is not being eaten, as the incisors are constantly being ground away (a process known as rodent bruxism) despite conditions of experimental hypodynamia.

In designing the experiments for a given level of significance ( $t \geq 2$ ;  $P \leq 0.05$ ), and based on the corresponding formulae (V. Yu. Urbakh, 1964; Hicks, 1967), we calculated injected radioactive isotope doses and



optimal numbers of experimental (10) and control (15) animals, and test analyses of the tissues under study, the optimal counting rate during radiometry of samples, and other experimental parameters. In all, 504 tests on calcified tissues were analyzed (three times). The numerical data obtained were statistically worked up using Student's method. The results were summarized on graphs showing percents of control values (see figures).

## Results

$\text{Ca}^{45}$  uptake in the molars on the 15th day of the experiment was somewhat elevated -- 8.4%, but this discrepancy was not significant ( $P > 0.05$ ). On the 30th day, its content significantly ( $P < 0.01$ ) decreased 17%.  $\text{Ca}^{45}$  uptake was again increased 7.8% ( $P > 0.05$ ) on the 60th day of the experiment. Only at the conclusion of the studies, on the 100th day, did a statistically significant ( $P < 0.001$ ) decrease in  $\text{Ca}^{45}$  uptake occur in the molars of the experimental animals -- 15.6% relative to controls.  $\text{Ca}^{45}$  uptake in the MF of the incisors was significantly ( $P < 0.001$ ) elevated by 18% after 15 days, and 11% ( $P < 0.02$ ) on the 30th day. On the 60th day of the experiment,  $\text{Ca}^{45}$  content in <sup>/4</sup> the incisors of the experimental animals remained elevated -- by 9.1% (an insignificant discrepancy --  $P > 0.05$ ). After 100 days, incisor  $\text{Ca}^{45}$  decreased 2.7%; this discrepancy is within limits for methodological error and is not significant ( $P > 0.05$ ).

In the bones of the lower jaw,  $\text{Ca}^{45}$  levels fluctuated within normal limits on the 15th day of the experiment; the 3% increase was not significant ( $P > 0.05$ ). On the 30th day,  $\text{Ca}^{45}$  uptake was reduced 17% ( $P < 0.001$ ). On the 60th and 100th days of the experiment,  $\text{Ca}^{45}$  uptake levels in the inferior maxillae were decreased significantly, by 11.5 and 19.4% ( $P < 0.001$ ) respectively. More abrupt and pronounced regularities were found in the femurs;  $\text{Ca}^{45}$  uptake in the MF of these bones was reduced to a significant extent over the entire course of the experiment, by 11-21% relative to the controls (see figure, B).

$P^{32}$  uptake indices in the MF of the molars, incisors, and inferior maxillary and femoral bones turned out to be decreased 7-33% relative to the controls in these assorted tissues. (see figure, A).

$P^{32}$  uptake in the PF of the calcified tissues was rather different than in the MF. In the PF of the incisors, molars, and femurs it changed in two consecutive phases. In the initial period of the experiment,  $P^{32}$  uptake levels in the PF of these tissues was elevated 15 14-30% relative to the controls. After 100 days, this uptake level was reduced 13.8-33.5% relative to the controls.  $P^{32}$  uptake in the PF of the lower jaw bones was low over the entire period of the experiment. The percent

Uptake of  $P^{32}$  in the mineral (A) and protein (C) fractions, of  $Ca^{45}$  in the mineral (B) and  $2C^{14}$ -glycine (D) in the protein fractions of calcified tissue during experimental hypodynamia.

m -- molars; i -- incisors; m -- inferior maxillary bones; f -- femurs. Circles designate non-significant discrepancies, dots show significant discrepancies at  $n = 45$ .

of reduction was increased from 7 on the 15th to 23.7 on the 100th day of the experiment (see figure, C).

The characteristics of  $2C^{14}$ -glycine uptake were dissimilar in the PF of differing calcified tissues. In the PF of the molars, its uptake was increased over the entire period (by 9-70%).  $2C^{14}$ -glycine content in the PF of the incisors changed in two stages: in the first stage (up to the 60th day), a 13-16% increase was found, while in the second there were 15.1% (60th day) and 5.9% (100th day) decreases.  $2C^{14}$ -glycine content in the PF of the inferior maxillary and femoral bones was reduced 22.5-35.5%, relative to the controls, over the entire period of the experiment (see figure, D).

## Discussion of Results

A decrease in metabolic processes in calcified tissues was found during the experimental hypodynamia of the research animals, particularly at the end of the experiments (on the 100th day). The most pronounced changes were found in  $P^{32}$  and  $2C^{14}$ -glycine metabolisms, the more labile (than  $Ca^{45}$ ) indices of mineral and protein metabolism in calcified tissues. The less pronounced alterations of  $Ca^{45}$  metabolism were, apparently, absolutely regular and accountable, since this element is a more "stable" metabolite than  $P^{32}$ .

Metabolic disorders occurring during hypodynamia were considerably more complex than previously indicated (by Hatter and McMillan, for example), and have a temporal relationship to the principally acting factor of hypodynamia.

The experiments showed that the nature and tendency of changes in the mineral metabolism of the various labeled compounds differed for this or that calcified tissue. For example, two-phase alterations in  $P^{32}$  and  $2C^{14}$ -glycine were manifested in the proteins of the incisors and femoral bones. It is interesting to note that pronounced changes in metabolic processes also arose in the bones of the lower jaw, which maintained its usual mobility even during hypodynamia. The same could be said in regard to the teeth, both the upper, which are motionless altogether, and the lower, which are motionless relative to the body of the lower jaw.

The experiments we conducted not only confirmed our working hypothesis, but also considerably expanded the existing conception of the question at hand. Disorders of mineral metabolism were accompanied by extremely complicated and interrelated alterations in mineral and protein metabolisms in the calcified tissues during experimental hypodynamia. This can be seen particularly graphically when comparing  $P^{32}$  uptake in the mineral and protein fractions of the calcified tissues; here the differing tendencies of alteration in uptake of this element in the differing fractions -- mineral and protein -- of the calcified

tissues were shown. Apparently,  $P^{32}$  is being redistributed among the differing fractions of the calcified tissues, and this was manifested also in other forms of pathology, such as dental caries and radiation lesions of the bones (A. A. Prokhonchukov, N. A. Zhizhina, 1967).

The alterations in metabolic processes manifested during experimental hypodynamia are quite reminiscent of metabolic disorders occurring during experimental dental caries, parodontopathies, spontaneous atrophy of the alveolar process of the lower jaw, radiation lesions of calcified tissues, and, particularly, developmental alterations in the metabolism of calcified tissue (N. A. Fedorov et al., 1964; Yu. A. Fedorov, 1964; A. A. Prokhonchukov and N. A. Zhizhina, 1967). We should emphasize that a seemingly premature aging of the body, with the characteristic alterations in metabolic processes, occurs during experimental hypodynamia (I. A. Arshavskiy, 1967; Ye. V. Farina, 1967).

### Conclusions

The results of these experiments gave evidence that it is not simple quantitative metabolic shifts, but extremely complex qualitative alterations in metabolic processes that arise in the calcified tissues during experimental hypodynamia.

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